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'LAETITIA' PLUMS STORED IN CONTROLLED ATMOSPHERES COMBINED WITH INDUCTION OF MASS LOSS AND ETHYLENE MANEGEMENT[1]

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ABSTRACT - Two experiments were conducted to evaluate the relative effects of controlled atmosphere (CA) associated with 1-methylcyclopropene (1-MCP; 1.0 μL L^{-1}), induction of mass loss (IML; 2%), and low ethylene (LE; <0.04 μL L^{-1} of C_2H_4) on the quality preservation of 'Laetitia' plums. In experiment 1 (2010), the treatments evaluated were cold storage (CS; 21.0 kPa O_2 + <0.03 kPa CO_2), CA1 (1 kPa O_2 + 1 kPa CO_2), CA1 + 1-MCP, CA1 + IML, and CA1 + LE. In experiment 2 (2011), the treatments evaluated were CS, CA2 (2 kPa O_2 + 2 kPa CO_2), CA2 + IML, and CA2 + 1-MCP. In both experiments, the fruit were stored at 0.5 ± 0.1°C and 96 ± 2% RH. CA storage delayed fruit ripening in both atmosphere conditions evaluated and reduced the internal browning of the 'Laetitia' plums, particularly in CA2. 1-MCP, LE, and IML had additional effects to CA1 on preserving flesh consistency. 1-MCP, irrespective of the CA condition, and IML, in CA1, reduced internal browning. CA1, regardless of the complementary technologies, reduced the incidence of decay and fruit cracking.

Keywords: *Prunus salicina*. Internal browning. Physiological disorder. Ripening.

AMEIXAS 'LAETITIA' ARMAZENADAS EM ATMOSFERA CONTROLADA COM INDUÇÃO DE PERDA DE MASSA E MANEJO DO ETILENO

RESUMO - Foram conduzidos dois experimentos com o objetivo de avaliar o efeito da atmosfera controlada (AC) associada ao 1-metilciclopropeno (1-MCP; 1,0 μL L^{-1}), à indução de perda de massa (IPM; 2%) e ao baixo etileno (BE; <0,04 μL L^{-1} de C_2H_4) sobre a manutenção da qualidade de ameixas 'Laetitia'. No experimento 1, em 2010, os tratamentos avaliados foram armazenamento refrigerado (AR; 21 kPa O_2 + <0,03 kPa CO_2), AC1 (1 kPa O_2 + 1 kPa CO_2), AC1+1-MCP, AC1+IPM e AC1+BE. No experimento 2, em 2011, os tratamentos foram AR, AC2 (2 kPa O_2 + 2 kPa CO_2), AC2+1-MCP e AC2+IPM. Em ambos os experimentos, os frutos foram armazenados a 0,5±0,1 ºC e 96±2% de umidade relativa. A AC retardou o amadurecimento, em ambas as atmosferas avaliadas, e reduziu o escurecimento de polpa de ameixas 'Laetitia', especialmente na AC2. O 1-MCP, o BE e a IPM apresentaram efeito adicional à AC1 na manutenção da consistência da polpa. O 1-MCP, independente da condição de AC, e a IPM, na condição de AC1, reduziram o escurecimento da polpa. A AC1, independente do uso de tecnologias complementares, reduziu a incidência de podridões e de rachaduras.

Palavras-chave: *Prunus salicina*. Escurecimento da polpa. Distúrbio fisiológico. Amadurecimento.

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INTRODUCTION

Internal browning is a process known as cold damage that affects stored plums (SINGH; SINGH, 2013a, b). This process is caused by an oxidative process related to production of reactive oxygen species and reduction of efficiency of antioxidant systems, with consequent damages to cell membranes (SINGH; SINGH, 2012; 2013a, b).

Controlled atmosphere (CA) prolongs the conservation of 'Laetitia' plums (STEFFENS et al., 2013; 2014). According to Steffens et al. (2014), atmospheric conditions with 1 kPa O_2+1 kPa CO_2 and 2 kPa O_2+2 kPa CO_2 are considered the best conditions for 'Laetitia' plum storage. However, even under these conditions, internal browning of 'Laetitia' plums may still occur (STEFFENS et al., 2013; 2014).

Internal browning may be aggravated by the effect of ethylene (CANDAN; GRAEL; LARRIGAUDIÉRE, 2011; CORRÊA et al., 2011) and lower diffusion of O_2 and CO_2 gases through the flesh in stored fruits (BRACKMANN et al. 2014). Thus, the use of complementary technologies to CA, such as 1-methylcyclopropene (1-MCP), induction of mass loss (IML) and low ethylene (LE) may be alternatives to reduce the occurrence and severity of internal browning.

Several studies have shown the effect of 1-MCP on ripening delay and post-harvest fruit quality maintenance (CANDAN; GRAEL; LARRIGAUDIÉRE, 2011; CORRÊA et al., 2011; MANGANARIS et al., 2008). Since internal browning in plums is aggravated by ethylene, 1-MCP may reduce its occurrence (CANDAN; GRAEL; LARRIGAUDIÉRE, 2011; CORRÊA et al., 2011). On the other hand, CA combined with LE may present similar effects to 1-MCP in 'Laetitia' plums.

Diffusion of gases through fruit flesh is essential to prevent an excessive decrease in O_2 and increase in internal CO_2 levels, which may affect the postharvest quality maintenance (BRACKMANN et al., 2014). Therefore, IML can probably contribute to reduce internal browning of stored plums in CA, since this technique possibly results in the removal of water from intercellular spaces of the fruit flesh tissue, facilitating the diffusion of gases through the flesh to the storage atmosphere. In cold storage (CS), IML contributes to reduce internal browning in Laetitia plums (ALVES et al., 2009), however, the effect of this technique on plums stored in CA was not yet evaluated.

Some studies were carried out on 'Laetitia' plums stored in CA (ALVES et al., 2010a; STEFFENS et al., 2013; 2014), however, they aimed to identify ideal conditions of O_2 and CO_2 for storage. Evaluations of complementary techniques, such as 1-MCP, LE and IML, combined with ideal CA conditions, on storage of 'Laetitia' plums were not yet conducted, except for 1-MCP. However, the positive results of IML and LE in fruits in CS, especially on internal browning (ALVES et al., 2009), indicate a potential synergy between these technologies and CA to control fruit ripening and reduce internal browning.

The objective of this work was to evaluate the effect of CA, with ideal O_2 and CO_2 conditions, combined with application of 1-MCP, IML and LE, on postharvest quality maintenance of 'Laetitia' plums.

MATERIAL AND METHODS

The 'Laetitia' plum fruits were harvested at commercial harvest stage, i.e., when 50% of the fruit epidermis was red, in 2010 (Experiment 1) and 2011 (Experiment 2) in a commercial orchard in Vacaria, State of Rio Grande do Sul, Brazil (28º40'48.65"S and 50º47'11.42"W, average altitude of 973 m). The fruits were taken to a laboratory and defective fruits were discarded, then, experimental samples were homogenized and placed in 0.45m^3 experimental micro-chambers, which were inside 45m^3 cold rooms, to applying the treatments.

The treatments of Experiment 1 were cold storage (21 kPa O_2+ CO_2<0.03 kPa) (CS), controlled atmosphere (CA1) (1 kPa of O_2+1 kPa of CO_2), CA1 combined with 1-methylcyclopropene (1 μL L^{-1}) (CA1+1-MCP), CA combined with induction of mass loss (2%) (CA1+IML) and CA1 combined with low ethylene (CA1+LE). The average ethylene concentration in the storage atmosphere of the treatments was 5.23 (CS), 1.12 (CA1) and <0.04 (CA1+LE) μL L^{-1}. The treatments of Experiment 2 were CS, CA2 (2 kPa of O_2+2 kPa of CO_2), CA2+1-MCP (1 μL L^{-1}) and CA2+IML (2%). The fruits of both experiments were stored at 0.5±0.1 °C and 96±2% of relative humidity.

Partial gas pressures were obtained by diluting O_2 in the micro-chambers with N_2 injection from a N_2 separator that uses the pressure-swing adsorption principle. Daily analyses were carried out for the maintenance of partial gas pressures in the chambers, using an automatic gas control device (Kronenberger Climasul). The excess CO_2 was absorbed by a potassium hydroxide solution (40%), through which the gases from the micro-chambers were circulated.

IML was induced by a constant air humidity absorption in the micro-chamber, in order to achieve a fresh mass loss of 2% at the end of storage period. This procedure was carried out with a membrane pump, which circulates air from the micro-chamber interior to a container containing silica gel, according to the methodology described by Brackmann et al. (2007).

Removal of ethylene in the treatment low ethylene (LE) (ethylene<0.04 μL L^{-1}) was carried out

with chemical absorption by adding packets containing potassium permanganate (one packet for each 3 kg of fruits) in the micro-chamber. Ethylene levels were monitored weekly by injecting 1 mL of gas from the chamber atmospheres into a gas chromatograph device (Varian® 3400CX, Palo Alto, CA, USA), equipped with flame ionization detector and a 0.7-m long Porapak N® column, using nitrogen as carrier gas. The temperatures used were 90 °C (column), 140 °C (injector) and 200 °C (detector).

The product SmartFresh® (0.14% of 1-MCP, powder formulation) was used for the treatment with 1-MCP, applying 1.6 g of this product for each m^3 of chamber to obtain 1 μL L^{-1} of 1-MCP. This product was placed in an airtight vial and solubilized in water at room temperature. Thereafter, the vial was introduced into the micro-chamber, and the solution was transferred to a Petri dish through a side opening and immediately sealed. The application of 1-MCP started one day after harvest and the fruits were subjected to this treatment for 24 hours.

The analyzes of the experiments were carried out after 55 days of storage and after 55 days of storage plus three days at room conditions (23±5 °C and 60±5% RH) (Experiment 1), and after 30 and 50 days of storage, after 30 days of storage plus 4 at room conditions, and after 50 days of storage plus 4 at room conditions (Experiment 2). The attributes evaluated were respiratory rate, ethylene production rate, flesh firmness, texture (pressures needed to epidermis rupture, flesh penetration and fruit compression), titratable acidity, soluble solids, red color index, epidermis color (hue angle, *h*°), occurrence and severity of internal browning and occurrence of rotting and cracking.

Respiratory rates and ethylene production rates were determined by placing 15 fruits of each sample in an airtight 2.3-L container and subsequently, gas samples (1000 μL) were taken from these containers through a septum and injected into a gas chromatograph device (Varian® CP-3800), equipped with a 3.0-m long Porapak N® column (80-100 mesh), methanator and flame ionization detector. Temperatures of 45 °C (column), 120 °C (detector), 300 °C (methanator), and 110 °C (injector) and flow of 70 mL min^{-1} (nitrogen), 30 mL min^{-1} (hydrogen) and 300 mL min^{-1} (synthetic air) were used. Respiratory rates were expressed as nmol of CO_2 kg^{-1} s^{-1} and ethylene production rates as pmol of C_2H_4 kg^{-1} s^{-1}.

Flesh firmness was determined (N) in the equatorial region of the fruits, on two opposite sides, after removal of a small portion of the epidermis, using a penetrometer equipped with a 7.9 mm diameter tip.

Titratable acidity (mEq $100mL^{-1}$) was evaluated in a 10-mL sample of juice prepared from transversal slices taken from the equatorial region of the fruits and ground in a centrifuge. This sample was diluted in 90 mL of distilled water and titrated with 0.1 N NaOH solution to pH 8.1.

Soluble solids content (°Brix) was determined by refractometry, using the juice extracted as described for titratable acidity, correcting the effect of temperature (20 °C).

Texture attributes (forces needed to epidermis rupture, flesh penetration and fruit compression) were analyzed in an electronic texturometer (TAXT-plus®, Stable Micro Systems Ltd., UK). The evaluation of forces needed to epidermis rupture and flesh penetration was carried out using a PS2 tip, which was introduced into the pulp at a depth of 5 mm with speeds of 30 (pre-test) 5 (test) and 30 (post-test) mm s^{-1}, without removal of the epidermis. The force for fruit compression was determined using a 75-mm diameter flat P/75 platform, applying increasing pressure up to a 5-mm deformation on the fruit surface.

The red color index of the fruit red surface was evaluated with a scale of grades according to the fruit percentage of red pigmented surface, 0-25% (1), 26-50% (2), 51-75% (3) and 76-100% (4). This index was calculated by dividing the number of fruits in each grade by the total number of fruits of the sample.

The epidermis color was determined with a colorimeter (Minolta CR400) and readings were performed on the more and less red sides of the fruit, in the equatorial region. The results were expressed in hue angle (*h*°). The *h*° defines the basic coloration as 0° = red, 90° = yellow and 180° = green.

The occurrence of rotting fruits was evaluated by counting affected fruits, which had lesions larger than 5 mm in diameter and characteristics of pathogen attack.

The occurrence of internal browning was visually evaluated on cross sections of the equatorial region of the fruit, by counting the fruits that showed internal browning, with results expressed in percentages (%).

The severity of internal browning was evaluated with a colorimeter (Minolta CR 400), in the median region of the fruit pulp, with results expressed in luminosity (*L*), from 0 (black) to 100 (white).

A completely randomized experimental design, with four replications was used for Experiment 1 (experimental units consisting of 20 fruits), and five replications for Experiment 2 (experimental units consisting of 40 fruits). Data were subjected to analysis of variance (ANOVA). Data expressed in percentages were transformed by the arcsen formula $[(x+0.5)/100]^{1/2}$ before subjected to ANOVA. Tukey's test was used to compare the means of treatments ($p<0.05$).

RESULTS AND DISCUSSION

Experiment 1 – 2010

The harvested fruits showed 50% of the epidermis surface covered with red color (Index 2), soluble solids (SS) of 14.0 °Brix, titratable acidity (AT) of 28.2 meq 100 mL^{-1}, flesh firmness of 49.9 N, pressures needed to epidermis rupture of 10.8 N, flesh penetration of 3.7 N and fruit compression of 113.6 N.

After 55 days of storage plus three days at room conditions, the respiratory rate of the treatments was similar (data not shown).

The flesh firmness and texture attributes (forces to epidermis rupture, flesh penetration and fruit compression) of all treatments in CA1, at withdrawing from the chambers (55^{th} day of storage), were higher than those of fruits in CS. However, after three days at room conditions, fruits stored in CA1 combined with 1-methylcyclopropene (1-MCP), induction of mass loss (IML) and low ethylene (LE) maintained their flesh firmness and texture attributes higher than those of fruits in CS and CA1 (Table 1). The maintenance of flesh firmness of fruits in CA1+1-MPC was related to the effect of 1-MCP on the inhibition of ethylene action, since ethylene promotes activity of enzymes responsible for fruit softening (CANDAN, GRAEL; LARRIGAUDIÉRE, 2011; KHAN; SINGH, 2007). Alves et al. (2009, 2010b), Candan, Grael and Larrigaudiére (2011) and Manganaris et al. (2008), also observed delay in flesh firmness loss in plums due to the effect of 1-MCP, corroborating the results of the present work.

The greater flesh firmness of the fruits stored in CA1 with LE was due to the lower action of ethylene in the activation of enzymes responsible for fruit softening. A greater flesh firmness in fruits subjected to IML was also found in 'Gala' apples stored in CA (BRACKMANN et al., 2007) and 'Laetitia' plums in CS (ALVES et al., 2010b). IML caused a lower production of ethylene in 'Gala' apples, which may explain the greater flesh firmness of fruits stored with IML (BRACKMANN et al., 2007).

Table 1. Flesh firmness and texture attributes (forces to epidermis rupture, flesh penetration and fruit compression) of 'Laetitia' plums under cold storage (21 kPa O_2+ CO_2<0.03 kPa) (CS), controlled atmosphere (CA1) (1 kPa of O_2+1 kPa of CO_2), CA1 combined with 1-methylcyclopropene (1 µL L^{-1}) (CA1+1-MCP), CA combined with induction of mass loss (2%) (CA1+IML) and CA1 combined with low ethylene (ethylene<0.04 µL L^{-1} de C_2H_4) (CA1+LE), after 55 days of storage (0.5±0.1 ºC; 96±2% RH) and after 55 days of storage plus three days at room conditions (23±5 °C; 60±5% RH).

Treatments	Flesh firmness (N)	Force to epidermis rupture (N)	Force to flesh penetration (N)	Force to fruit compression (N)
	After 55 days of storage (chambers opening)			
CS	22.53b*	4.53b	1.12b	52.38b
CA1	41.44a	10.25a	2.59a	131.48a
CA1 + 1-MCP	40.12a	9.98a	2.56a	133.97a
CA1 + IML	39.29a	10.60a	2.48a	137.23a
CA1 + LE	39.24a	9.50a	2.38a	149.26a
CV (%)	10.30	8.89	16.44	8.20
	After 55 days of storage plus three days at room conditions			
CS	26.69c	4.48d	1.05b	70.54d
CA1	29.68c	7.87c	1.25b	125.93c
CA1 + 1-MCP	42.03a	10.97a	2.15a	181.48a
CA1 + IML	35.79b	9.76b	1.94a	164.69b
CA1 + LE	38.56ab	10.24b	2.16a	176.01ab
CV (%)	11.50	4.82	11.67	6.07

*Means followed by the same letter in the columns do not differ by Tukey's test ($p<0.05$).

Fruits in CA1 showed higher TA at withdrawing from the chambers than those in CS, regardless of the use of complementary techniques. After three days at room conditions, fruits in CA1+1-MCP had higher TA than those in CA1 and CS, but they did not differ from those in CA1+IML and CA1+LE (Table 2). Alves et al. (2010a) and Argenta et al. (2003) also found higher TA in 'Laetitia' plums treated with 1-MCP.

Table 2. Titratable acidity, soluble solids and red color index (RCI) of 'Laetitia' plums under cold storage (21 kPa O_2+ CO_2<0.03 kPa) (CS), controlled atmosphere (CA1) (1 kPa of O_2+1 kPa of CO_2), CA1 combined with 1-methylcyclopropene (1 µL L^{-1}) (CA1+1-MCP), CA combined with induction of mass loss (2%) (CA1+IML) and CA1 combined with low ethylene (ethylene<0.04 µL L^{-1} de C_2H_4) (CA1+LE), after 55 days of storage (0.5±0.1 ºC; 96±2% RH) and after 55 days of storage plus three days at room conditions (23±5 ºC; 60±5% RH).

Treatments	Titratable acidity (meq 100mL^{-1})	Soluble solids (Brix)	Red color index (RCI) – (1-4**)
After 55 days of storage (chambers opening)			
CS	11.9b*	9.4a	3.6a
CA1	18.6a	8.9ab	2.8b
CA1 + 1-MCP	18.4a	9.1ab	2.6b
CA1 + IML	18.1a	8.8ab	2.9b
CA1 + LE	18.6a	8.5b	2.9b
CV (%)	12.7	6.0	8.8
After 55 days of storage plus three days at room conditions			
CS	11.3c	8.8a	3.8a
CA1	15.0b	7.3b	3.5b
CA1 + 1-MCP	18.0a	7.4b	3.3c
CA1 + IML	17.6ab	7.2b	3.5b
CA1 + LE	15.5ab	7.0b	3.3c
CV (%)	12.5	10.1	4.8

*Means followed by the same letter in the columns do not differ by Tukey's test ($p<0.05$).
**Red color index (RCI): 1 = 0 to 25% red; 2 = 26 to 50% red; 3 = 51 to 75% red; 4 = >75% red.

The SS contents of the fruits in CS at withdrawing from the chamber were significantly different than those in CA1+LE. However, after three days at room conditions, the fruits in CA1, showed lower SS, regardless of the use of complementary techniques (Table 2). The treatments in which flesh firmness and texture attributes of fruits were lower, in general, had higher SS. The highest SS in CS are probably related to a higher content of soluble pectins, since these fruits had the lowest flesh firmness, confirming previous results that showed this inverse relationship between flesh firmness and SS content in 'Laetitia' plum (STEFFENS et al., 2011).

The red color index (RCI) of the fruits in CA1 at withdrawing from the chamber was lower compared with those stored in CS. After three days at room conditions, fruits in CA1+1-MCP and CA1+LE had the lowest RCI (Table 2).

The results of epidermis color ($h°$) was similar to the RCI (data not shown). Candan, Grael and Larrigaudiére (2011) also found a delayed epidermis color evolution in 1-MCP in plum cultivars with climacteric peak of ethylene production, but not in cultivars with suppression of climacteric peak of ethylene production. The effect of 1-MCP and ethylene absorption on the maintenance of the plum epidermis coloration, combined with the results of Candan, Grael and Larrigaudiére (2011), show the epidermal color

evolution of plums as a process dependent on the action of ethylene.

The occurrence of internal browning was high in all treatments (>87%), however it was lower in the fruits in CA1 and CA1+1-MCP at withdrawing from the chamber. However, after three days at room conditions, the treatments showed no difference (Table 3). Internal browning is a process that can occur at low temperatures and long periods of storage, which seems to be aggravated by the action of ethylene (CANDAN; GRAEL; LARRIGAUDIÉRE, 2011).

The high occurrence of pulp browning in all treatments may be related to the prolonged storage period. Singh and Singh (2013a) also found a higher occurrence of internal browning in plums over the storage period. Steffens et al. (2014), evaluated several CA and found high occurrence of internal browning in 'Laetitia' plums stored for 60 days, regardless of the CA conditions.

Table 3. Occurrence and intensity of internal browning, rotting and cracking of 'Laetitia' plums under cold storage (21 kPa O_2+ CO_2<0.03 kPa) (CS), controlled atmosphere (CA1) (1 kPa of O_2+1 kPa of CO_2), CA1 combined with 1-methylcyclopropene (1 µL L^{-1}) (CA1+1-MCP), CA combined with induction of mass loss (2%) (CA1+IML) and CA1 combined with low ethylene (ethylene<0.04 µL L^{-1} de C_2H_4) (CA1+LE), after 55 days of storage (0.5±0.1 ºC; 96±2% RH) and after 55 days of storage plus three days at room conditions (23±5 °C; 60±5% RH).

Tratamentos	Internal browning (%)	Internal browning intensity (*L*)	Rotting (%)	Cracking (%)
After 55 days of storage (chambers opening)				
CS	100.0a*	40.1c	50.0a	82.5a
CA1	88.5b	43.3b	1.9c	0.6b
CA1 + 1-MCP	87.3b	46.2a	2.8c	0.7b
CA1 + IML	91.7ab	48.5a	17.7b	0.6b
CA1 + LE	95.0ab	43.2b	2.5c	0.6b
CV (%)	13.2	4.0	30.1	30.3
After 55 days of storage plus three days at room conditions				
CS	100.0a	40.3c	36.1a	93.7a
CA1	93.4a	40.5c	22.1a	0.1b
CA1 + 1-MCP	96.1a	45.6a	15.1a	1.3b
CA1 + IML	97.4a	45.7a	10.7a	0.1b
CA1 + LE	96.3a	43.2b	19.2a	0.1b
CV (%)	9.3	3.7	40.1	33.2

*Means followed by the same letter in the columns do not differ by Tukey's test ($p<0.05$).

The internal browning intensity (evaluated in terms of color parameter *L*) was lower in fruits in CA1+1-MCP and CA1+IML at withdrawing from the chamber, and also after three days at room conditions (Table 3). According to Corrêa et al. (2011), when the 'Laetitia' plum internal browning is at an early stage, these fruits may have good acceptability by consumers, and fruit flesh with *L* of 48.7 had an acceptability above 50%, however, fruit flesh with *L* of 41.3 had 95% of rejection.

The fruits in CA1+IML had *L* of 48.5 (at withdrawing from the chamber) and 45.6 (after three days at room conditions). The fruits in CA1+1-MCP had *L* of 46.2 (at withdrawing from the chamber) and 45.6 (after three days at room conditions). These results of *L* were close to those observed by Corrêa et al. (2011), who found reasonable acceptability for the fruits. The fruits in CS and CA1 had *L* ≤41.3 after three days at room conditions, which according to Corrêa et al. (2011) would have high rejection by

consumers.

All fruits in treatments with CA1 had lower occurrence of rotting and cracking at withdrawing from the chamber than those in CS. However, after three days at room conditions, there were differences in cracking, with lower occurrence in fruits from treatments with CA1 (Table 3). This lower cracking occurrence in CA1 was related to the less advanced maturity stage of fruits in CA1 compared with those in CS. Steffens et al. (2014) also found lower occurrence of rotting and cracking in fruits in CA1 compared with fruits in CS. The effect of CA on the reduction of rotting can be attributed to the low levels of O_2 combined with high levels of CO_2, which have a fungistatic effect, inhibiting spore germination and fungus growth during the storage period (VIEIRA et al., 2006).

Experiment 2 – 2011

The harvested fruits showed 50% of the epidermis surface covered with red color (Index 2), SS of 14.8 °Brix, TA of 26.0 meq 100 mL^{-1}, flesh firmness of 51.5 N, pressures needed to epidermis rupture of 11.7 N, flesh penetration of 3.4 N, and fruit compression of 114.2 N.

All fruits in CA2 had lower ethylene production rate than those in CS in all evaluation times (at withdrawing from the chambers after 30 and 50 days of storage, after 30 days of storage plus 4 at room conditions, and after 50 days of storage plus 4 at room conditions) (Table 4). The ethylene production in CA conditions was low due to the low partial pressure of O_2 caused by low oxidation of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (BLANKENSHIP; DOLE, 2003). Moreover, the high partial pressure of CO_2 reduces cell pH, inhibiting the activity of the enzyme ACC oxidase, contributing to reduce ethylene biosynthesis (GORNY; KADER, 1996). The treatments CA2, CA2+IML and CA2+1-MCP were similar in ethylene production in all evaluation periods.

According to Gorny and Kader (1996), the low partial pressure of O_2 reduces the respiratory activity. However, after 30 days of storage at withdrawing from the chambers, and also after four days at room conditions, the respiratory rate of fruits in CA2+1-MCP was lower than those in fruits in CS, but not differing from the other treatments in CA2. After 50 days of storage, the respiratory rate of fruits was similar in all treatments, and after four days at room conditions, fruits in CA2 had lower respiratory rates than those in CS. These results show the effect of 1-MCP on preventing the binding of ethylene to the receptor, reducing the action of ethylene on the enzymes that act in the respiratory process.

Flesh firmness of all fruits in CA2 was, in general, higher than those in CS. The higher loss of flesh firmness in CS was related to the high ethylene production in fruits in this treatment. Corrêa et al. (2011) evaluated Laetitia plums and also found lower flesh firmness in treatments with higher ethylene production. Ethylene promotes the activity of enzymes responsible for fruit softening (CANDAN; GRAEL; LARRIGAUDIÉRE, 2011; KHAN; SINGH, 2007).

The results of pressures needed for epidermis rupture, pulp penetration and fruit compression were similar those of flesh firmness (data not shown).

The red color index (RCI) of all treatments after 30 days of storage were similar (Table 4). However, after four days at room conditions, fruits in CA2+1-MCP had lower index than those in CS, but not differing from those in CA2+IML (Table 4). After 50 days of storage, the lowest RCI was found in fruits in CA2+IML, but not differing from those in CA2 (Table 4).

After 50 days of storage plus four days at room conditions, all fruits in CA2 had lower RCI than those in CS (Table 4). The results of epidermis color ($h°$) was similar to those of RCI, showing no difference after the 30 days of storage. Fruits of the treatments in CA2 had, in general, higher $h°$, i.e., less red color compared with fruits in CS (data not shown). The higher color evolution in CS may be related to the higher ethylene production of fruits in CS during storage. According to Argenta et al. (2003), processes responsible for color change in plums are dependent on ethylene. However, fruits in CA2+1-MCP showed no consistent differences to the other CA2 treatments. Similar results were found by Corrêa et al. (2011).

The highest occurrence and severity of internal browning in all evaluations was found in fruits in CS, which also had the lowest pulp luminosity (L), indicating a greater intensity of internal browning (Table 5). Singh and Singh (2013a) and Steffens et al. (2014) also found lower internal browning in fruits in CA compared with fruits in CS. The effect of CA on reducing internal browning is related to the increase of antioxidant metabolism and suppression of the oxidative process (SINGH; SINGH, 2013a).

Occurrence and severity of internal browning in fruits in CA2 after 30 days of storage was similar.

Table 4. Respiratory rate, ethylene production, pulp firmness and red color index (RCI) of 'Laetitia' plums under cold storage (21 kPa O_2+ CO_2<0.03 kPa) (CS), controlled atmosphere (CA2) (2 kPa of O_2+2 kPa of CO_2), CA1 combined with 1-methylcyclopropene (1 μL L^{-1}) (CA2+1-MCP) and CA combined with induction of mass loss (2%) (CA2+IML), after 30 and 50 days of storage (0.5±0.1 ºC; 96±2% RH) and plus three days at room conditions (23±5 °C; 60±5% RH).

Treatments	Ethylene production (ρmol C_2H_4 kg^{-1} s^{-1})	Respiratory rate (ηmol CO_2 kg^{-1} s^{-1})	Flesh firmness (N)	RCI (1-4)
	After 30 days of storage plus (chambers opening)			
CS	21.9a*	351.1a	41.3b	2.4a
CA2	2.3b	246.0ab	46.9ab	2.5a
CA2+IML	1.6b	225.3ab	46.8ab	2.4a
CA2+1-MCP	2.7b	202.1b	49.8a	2.5a
CV (%)	70.8	27.6	7.3	7.7
	After 30 days of storage plus four days at room conditions			
CS	53.7a	368.4a	8.3b	3.7a
CA2	2.0b	209.0ab	25.4a	3.1b
CA2+IML	2.5b	225.2ab	26.9a	3.0bc
CA2+1-MCP	2.0b	183.6b	29.4a	2.9c
CV (%)	126.2	39.8	13.6	4.0
	After 50 days of storage (chambers opening)			
CS	ND***	347.6a	25.9b	2.8a
CA2	ND	269.1a	41.0a	2.3ab
CA2+IML	ND	258.7a	44.2a	2.3b
CA2+1-MCP	ND	213.6a	42.6a	2.4ab
CV (%)	-	45.5	14.1	9.9
	After 50 days of storage plus four days at room conditions			
CS	24.7a	247.1a	24.2b	3.0a
CA2	12.8b	128.2b	37.7a	2.4b
CA2+IML	16.6b	165.1b	36.4a	2.7b
CA2+1-MCP	14.4b	130.5b	41.1a	2.6b
CV (%)	55.9	25.7	14.6	9.7

*Means followed by the same letter in the columns do not differ by Tukey's test ($p<0.05$).

In general, the fruits in CA2+1-MCP had the lowest occurrence and severity of internal browning. Fruits in CA2+IML had lower occurrence and severity of internal browning than those in CA2 after 50 days of storage, but not differing from those in CA2+1-MCP (Table 5). Fruits in CA2, after 50 days of storage plus four days at room conditions, had differences in flesh luminosity, with fruits in CA2+1 -MCP having higher *L* compared with those in CA2 (Table 5). These results show that the use of 1-MCP promotes an additional positive result to CA in controlling internal browning occurrence. According to Argenta et al. (2003), Laetitia plums show internal browning mainly after 30 days of storage. The high occurrence of internal browning in CS may be related to the high rate of ethylene production in this treatment (Table 5).

The variables rotting and cracking showed similar results in all treatments (data not shown).

Table 5. Occurrence and severity of internal browning and internal browning intensity (*L*) of 'Laetitia' plums under cold storage (21 kPa O_2+ CO_2<0.03 kPa) (CS), controlled atmosphere (CA2) (2 kPa of O_2+2 kPa of CO_2), CA2 combined with 1-methylcyclopropene (1 μL L^{-1}) (CA2+1-MCP) and CA combined with induction of mass loss (2%) (CA2+IML), after 30 and 50 days of storage (0.5±0.1 ºC; 96±2% RH) and plus three days at room conditions (23±5 °C; 60±5% RH).

Treatments	Internal browning (%)	Severity of internal browning (1-5**)	Internal browning intensity (*L*)
After 30 days of storage (chambers opening)			
CS	39.4a*	0.97a	55.2b
CA2	21.0b	0.45b	59.8a
CA2+IML	20.2b	0.54b	60.9a
CA2+1-MCP	19.7b	0.45b	60.9a
CV (%)	19.3	8.8	2.0
After 30 days of storage plus four days at room conditions			
CS	66.9a	1.67a	53.8a
CA2	4.0b	0.29b	53.4a
CA2+IML	5.6b	0.28b	54.5a
CA2+1-MCP	1.5c	0.03c	54.9a
CV (%)	26.7	58.0	5.5
After 50 days of storage (chambers opening)			
CS	100.0a	2.61a	40.8b
CA2	77.1b	2.49a	53.2a
CA2+IML	55.2c	1.60b	54.1a
CA2+1-MCP	58.0c	1.43b	54.5a
CV (%)	13.2	6.6	3.1
After 50 days of storage plus four days at room conditions			
CS	100.0a	3.90a	49.3c
CA2	72.6b	3.10b	52.4b
CA2+IML	67.8b	2.89b	54.5ab
CA2+1-MCP	42.3c	1.33c	55.1a
CV (%)	11.2	10.1	2.6

*Means followed by the same letter in the columns do not differ by Tukey's test ($p<0.05$).
**Internal browning severity: 0 = fruits without internal browning, 1 = up to 10%, 2 = 11 to 30%, 3 = 31 to 50%, 4 = 51 to 80%, and 5 = more than 80% of flesh browning.

CONCLUSION

Controlled atmosphere delayed ripening in both evaluated atmospheres and reduced the internal browning of the 'Laetitia' plum, especially in the conditions of 2 kPa O_2+2 kPa CO_2.

Treatments with 1-MCP, induction of mass loss and low ethylene had additional effects to the controlled atmosphere (CA) (1 kPa of O_2+1 kPa of CO_2) on the maintenance of flesh consistency.

Treatments with 1-MCP, regardless of the CA condition, and treatment with IML in the atmosphere of 1 kPa of O_2+1 kPa of CO_2, reduced internal browning.

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